AUTOIMMUNE DISEASES: HEMOLYTIC ANEMIA

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Autoimmune Diseases: Hemolytic Anemia. (May 2014)

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Anemia is a term that defines a condition in which a person's blood lacks a stable amount of red blood cells or hemoglobin. This condition can cause a person to experience fatigue, and an improper amount of oxygen will be carried to the body and its organs. Hemolytic anemia is an autoimmune disease similar to anemia, which wages a battle against the body's own red blood cells. Hemo is derived from the Greek "Haema" meaning blood, and lytic from the Greek "loosing". Thus, even though a person with hemolytic anemia has actually experienced no physical blood loss, people infected with the disease see a decline in the amount of red blood cells in their body. To this day, there is no evidence on how or why a person's immune system is attacking the body's red blood cells. There are many theories, and experiments in this field are generating a vast pool of information to determine an eventual mechanism and hopefully a cure. My goal is to propose a hypothesis for the mechanism of this disease, and then hopefully be able to test it. Determination of a mechanism could hopefully lead to a treatment for this autoimmune disease, and if successful I will attain a solution.

DEDICATION

To my loving parents, for always believing me, and for making me the person I am today.

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I appreciate and will be forever thankful, to my research advisor. Dr. Aramayo believes in me and my life goals, and there is nothing else I could ever ask for. He challenges me, and made this entire research project possible. Thank you also, to the Research Scholars program. Thank you for your help, advice, and for the resources provided to us.

CHAPTER I

INTRODUCTION

Autoimmune diseases, hemolytic anemia

Inherent in this generation's strive to success, is the improvement of medicine and health in general. Humans today live an average life span unimaginable to people of the last century. Albeit that many countries around the world are still living in bad conditions, there is no doubt we are making progress for a better tomorrow.

Unfortunately, complications, failures and even regression are important factors to long term success. The cleanliness, within which we live, is surprisingly causing problems that have never been seen before. Infection is important to building resistance, and we are developing weak immune systems because they have never been exposed to bacteria or diseases. A new generation of medical problems is spreading quickly, and causing scientists to look into the future for solutions.

In specific, there is one umbrella of diseases that I am particularly interested in: autoimmune diseases. These diseases are a body's battle against itself. Being unable to recognize its own compartments, the body starts attacking itself through its immune system[1]. There are many different types and sub-types of autoimmune diseases, for most of which there is no explanation as to the mechanism or cause. In specific, the autoimmune disease I am interested in is hemolytic anemia.

Similar to anemia, hemolytic anemia involves the loss of blood from the body. Anemia can occur from physical loss of blood, destruction of red blood cells, and possibly from a defect in the process of producing red blood cells. Anemia usually leads to fatigue and a lack of oxygen throughout the body[2]. Hemolytic anemia is similar however; it is caused by a malfunction of the body's own immune system. The body recognizes the red blood cells as foreign, and begins to destroy them. Even though there is no physical blood loss in the patient, blood cells are being lost. Patients across the world are taken into hospitals or clinics, and after a lack of blood is diagnosed, simple transfusions are deemed sufficient. However, this is not the case and a deeper problem exists. Somewhere along the pathway that our immune system takes to combat foreign antigens, there is a step where our red blood cells are being destroyed.

Current research on autoimmune diseases is a growing field. Though it is an area of interest, there is still no explanation as to the mechanism of how hemolytic anemia arises. In my research for an answer, I have come across various literature reviews that have been an immense aid to me. First and foremost, there are several papers that have tried and explain how hemolytic anemia happens. One I find interesting is a review written by George Carratty. The review claims that not only are there hemolytic anemia due to immune system malfunctions, but hemolytic anemia can also be drug induced.

The reason this paper is of great importance to me, is because it gives us a way to test the mechanism for hemolytic anemia. The drugs mentioned in the paper are Heparin, Piperacillin, Cefotetan, and Ceftriaxone. If we can understand how these drugs induce hemolytic anemia, maybe we can understand how a virus or a bacterium can induce the body

to lyse its own blood cells. The way a drug induces this autoimmune response might not be the same as how a virus or bacteria does it however, it will still provide valuable information.

In another review, Segbjorn Berentsen and Geir E. Tjonnfjord disscuss the diagnosis and treatment of cold agglutinin mediated autoimmune hemolytic anemia. The review brings up an interesting theme, which revolves around the temperature dependence of hemolytic anemia. Cold agglutinin disease is an autoimmune disease in which lysis of red blood cells also occurs. However, it is only a subclass under the bigger branch called Autoimmune Hemolytic Anemia, and it occurs at low temperatures.

Through all my research, I have come to the conclusion that there is indeed more than one factor that causes hemolytic anemia. Several factors that are affecting our generation, and causing this autoimmune disease phenomenon include: the environment, hereditary factors, and pharmaceutical drugs (see figure above). SIAE gene is what I consider the underlying cause of hemolytic anemia. The SIAE gene is in charge of producing a very important enzyme in our body. This enzyme produces sialic acid, a carbohydrate that is placed on the surface of cells in the body to aid in recognition. My hypothesis is that these malfunctioning genes are producing an enzyme that is not labeling red blood cells properly, and they are being targeted by the body's immune system.

CHAPTER II

METHODS

Autoimmune diseases are a new phenomenon, that this and future generations will have to deal with. One can infer from the urgency that hemolytic anemia and other diseases are causing, that there is not much known about the topic. Our body is starting to attack itself, and neither doctors nor scientists fully comprehend why. Thus, my methods for conducting my research were mainly scholarly. My work consisted of compiling and analyzing literature on autoimmune diseases. The two major databases I used were PubMed, and UpToDate. Because of the fact that this is a fairly new topic, good literature on the subject was hard to find, especially anything that was actually relevant to my research. Interestingly, topics that are supposed to be related to hemolytic anemia are not always placed in the same category. Having to research unrelated fields made my research more interesting, and allowed me diversify my studying to include other subjects not in this field. There are so many valid explanations to this autoimmune phenomenon, including the use of food coloring in our food, the "Hygiene Theory", genetic predisposition and many more. At this point, any reason is valid, and all subjects in the field can possibly be related.

Very important to understand, is that a solution to this problem is still far away from now. I hope to contribute to the answer one day. I focused on two major contributing factors to autoimmune hemolytic anemia: the SIAE Gene, and pharmaceutical drugs. With each and every passing day, there are more and more synthetic drugs being made to fight off new disease. It almost seems like a never ending cycle, while one drug is made to fight a new disease, another disease suddenly emerges. By focusing on human made drugs and the interaction between them and gene products in our body, it allows me an opportunity to be

able to understand the mechanism by which the disease is happening. Scientists do not understand how the immune system is being triggered to attack its own blood cells, however we do understand the mechanisms by which drugs work. Consequently, in trying to connect the two pathways, and the point in which our immune system is breaking down, we might begin to understand autoimmune systems in greater detail.

What if we are causing our own diseases and we just don't know it yet? One drug in specific that I found interesting is Rituximan (Rituxuan). This drug is fairly new, and is used to treat an array of diseases dealing with white blood cells. Certain illnesses cause the number of B Cells in our body to reproduce uncontrollably. In a nutshell, Rituxuan destroys B Cells and reduces their numbers. The mechanism by which this is done involves the targeting of CD 20, a protein located on the surface of B-Cells [3]. I believe that in being able to understand the mechanism of this drug will also allow us to understand the mechanism by which our body is starting to attack itself. My research on this drug was scholarly, and I focused on reviews and experiments conducted by others. However, the human subjects that took the drug had very interesting responses to the drug in the reviews that I found. Some patients had the exact opposite desired response, and started making more B-cells than the body could handle[3]. Reactions like this lead to the unspecific attack of other types of cells in the body.

While scientists are making progress in this area, my main concern is that attacking B-cells is a very general approach to a very specific issue. Attacking B-Cells using the presence of a surface carbohydrate is the exact opposite of how our red blood cells are being destroyed. The lack of sialic acid is what I believe is destroying the red blood cells, and being able to focus on this issue would be a better usage of time and resources.

Our immune system is very complex. Not only does it keep us alive, but it also serves as a fort against the outside world. Very complex mechanisms, which are not fully understood, provide complex reactions that trigger our body to respond to foreign antigens. One of these pathways is being tempered with, and it is causing our body to attach its own red blood cells. Because this exact pathway, or the players involved is not known, exact research cannot be done at the moment on the actual problem. Methods that have been used have included a variety of different laboratory experiments, some of which revolve around the use of drugs.

Though I am not able to conduct research just yet, I would like to discuss some research methods that I believe would be beneficial to experts in this area. First off, it is a great possibility that human made drugs have triggered autoimmune responses, and have thus caused this phenomenon where our body destroys itself. By studying the mechanisms of these drugs, we will further being to understand the ways in which our immune system works.

Second, studies show that autoimmune susceptibility is genetic. Here is where genomics comes into play. By studying the genomes of healthy people, and people who are more susceptible to autoimmune diseases, perhaps an answer can be reached. If the sequencing of the SIAE gene can be done in a healthy human being, and then compared to the one in a patient with hemolytic anemia, an answer could be attained. In finding differences in the DNA sequences that relate to our immune system, we could perhaps find differences, and in the future perhaps even a cure.

There is one factor that is keenly working the background for all these diseases, and many sources blame our changing world for the rise in autoimmune diseases. In the last decades we have seen an increase in “cleanliness” and many rules and regulations for maintaining a

healthier world. Scientists attribute hemolytic anemia and other autoimmune diseases to the lack of exposure to certain bacteria[4]. Thus, studying cities in where cleanliness levels are high, and comparing them to "dirtier" cities, could allow for a comparison in the types of bacteria that have been lost in the process. The solution to hemolytic anemia could be as simple as the introduction of certain bacteria to young adults or babies. The lack of certain exposure and therefore a lack in immune defenses could be the explanation why later in life our bodies have such a disturbing reaction to our environment.

CHAPTER III

RESULTS

Autoimmune diseases are such a controversial topic coming into the 21st century. We have essentially driven our death rates down and extended our lifespan by improving our way of living and treatments for illnesses. However, it seems as if we are now leaving our bodies defenseless, and we are seeing a rise in autoimmune diseases. This topic is controversial for one reason: we don't know the answer to why our bodies are attacking themselves. There are some mechanisms that have been proposed, and there are a lot of dugs out there to combat these diseases. However, FDA approval on these drugs can sometimes be questionable because not enough long term research is being done.

My hopes when starting this project, were to produce a testable hypothesis for what could be causing this disease. Nonetheless, after much work, I feel that I have come up with a hypothesis for what could be causing hemolytic anemia. Up to this point, I have come to the conclusion that there is more than one factor affecting our human population. Though many scientists identify one factor as the main cause of autoimmune hemolytic anemia I disagree, in my opinion many factors are at play and they must be understood in order to be controlled. However, there is one main factor that underlies all of them and is the main cause of these diseases, and that is genetics.

As discussed earlier, our world is developing into a more hygienic world, and the first factor I would like to discuss is cleanliness. Society is becoming a cleaner and cleaner place. New methods, new medicines, and new innovations are helping us move away from the bacterial world. However, we have lived in an overlapping world with bacteria, and now that we are

trying to move away from this dynamic relationship, it is affecting our health. Ironically, we are becoming too clean for our own health. There have been actual studies that have mapped out major cities in America where the cleanliness level is high. In these same cities, there are increased levels of autoimmune diseases, and other rare and not-well known maladies[5].

Why is it then that not being exposed to certain bacteria at a young age, is affecting us in a negative way? Our immune system is like a castle built around our body, and it helps keep invaders out, and other bacteria at bay. We have many colonies living on the surface of our body at all times, and they help other bacteria stay away, and help keep us healthy. Our immune system is able to recognize certain bacteria after the second time that we are exposed to them. This is the idea behind flu shots. The idea is that you are injected with a very weak strain of the bacteria that causes flu, and once you are invaded by the real flu bacteria; your body will be able to recognize it. Once your body knows the nature of the invader, it will be able to generate an attack that much faster. Consequently, we are preventing our bodies from building up resistance to many bacteria that could be detrimental at a later stage in our lives.

The second factor is a continuation of the second. As we can tell by just taking a look at our surroundings, technology has come a very long way. We are able to do innovative things and explore areas of our world that we were not able to before. Consequently, with each new invention and each new breakthrough, we are trying to do the impossible and break barriers that have never been broken before. Just a couple of years ago we thought a cure for cancer was an impossible goal. However, scientists are now working hard and though we are not quite there yet, the future seems promising.

With this in mind, I establish the second issue. Scientists and large pharmaceutical companies are creating a lot of synthetic drugs that are designed to combat these "new world" diseases, and in some cases they are doing the exact opposite of their purpose. Of course the goal behind most of these drugs is a good one. They do indeed help a lot of people, and prevent deaths that seemed unlikely to be prevented. However, some of these drugs are very generic, and though they are very generic, they are still given to a lot of people regardless of their condition.

One of these drugs is Rituximab. As discussed earlier, this drug is an antibody that attaches to the surface of B cells, and essentially destroys them. This prevents the B-cells from destroying blood cells, and thus preventing hemolytic anemia. However, why attack our own immune cells? Why not attack the disease? Why not block receptor sites on blood cells that will prevent them from being recognized as foreign? I know this is easier said than done however, there are many cases in which this experimental drug is causing detrimental side effects that put people in a worse place than they were before. Death is common, and it seems that attacking our B-cells is a very generic solution to a very specialized type of disease. Consequently, we are slowly but surely breaking down our own immune systems, and opening the door for new autoimmune disease.

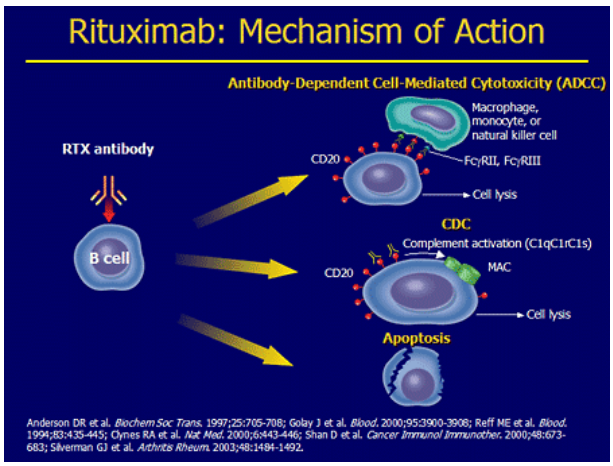

Figure 1 Rituximab Mechanism of Action

Next, we have genetic predisposition. It may not seem likely that some lineages are more susceptible to certain autoimmune diseases however, evidence is piling up that it is. Studies have been done on families in which certain diseases run through the family, and coincidence on autoimmune diseases is not likely the case. There is a hereditary factor, which scientists don't fully comprehend yet, that is added which is causing a predisposition to autoimmune diseases. Nonetheless, one of these factors by itself may not be strong enough to be the cause of an autoimmune disease. However, a combination of all of them is definitely a plausible explanation.

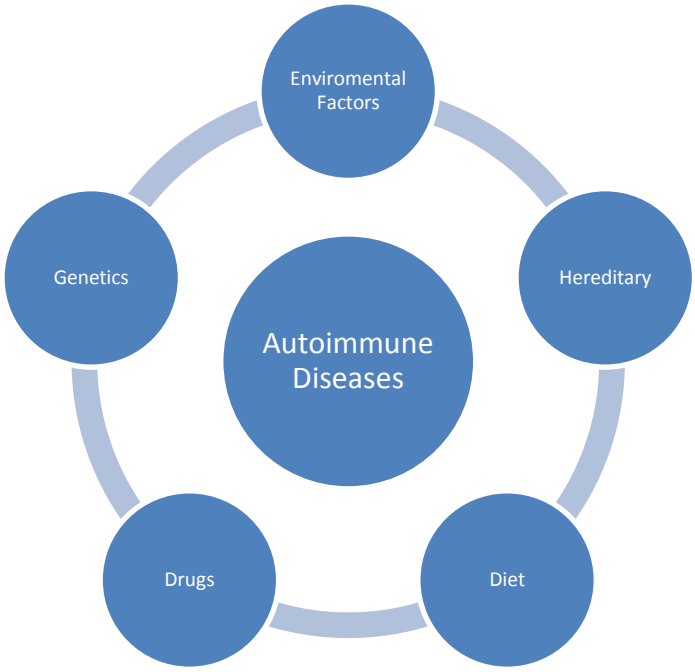

Figure 2 This figure shows the different factors that can have an effect on the chances of getting an autoimmune disease.

Finally, my hypothesis for what is causing hemolytic anemia revolves around the defect in the SIAE gene. The enzyme produced by this gene is in charge of creating sialic acid. This product is placed on the surface of cells throughout the body, which facilitates recognition. As a result, in patients with hemolytic anemia, this enzyme is not being created properly, and

this is causing red blood cells to lack sialic acid. The lack of sialic acid is causing red blood cells to appear foreign to our own immune system, which leads to an attack and depletion of red blood cells in the body. Thus, even though there is no actual physical blood loss, patients are diagnosed with low levels of hemoglobin. Blood transfusions are a popular answer to this problem, but are simply prolonging the true problem.

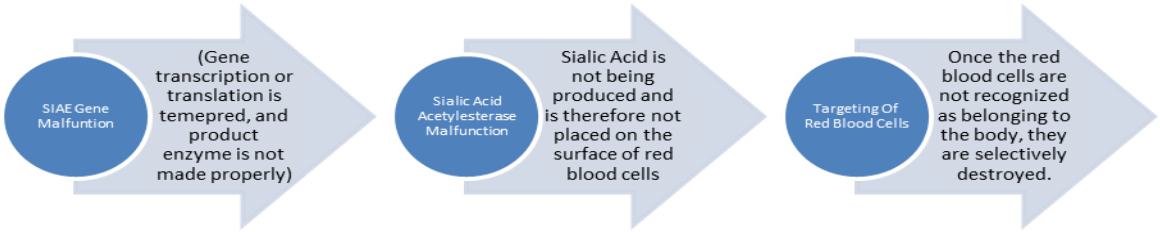

Figure 3 This figure explains my hypothesis about what I propose is going on in the case of patients with hemolytic anemia.

CHAPTER IV

CONCLUSION

All of my research has definitely led me to a new way of thinking about the bigger question, there are many diseases and issues out in the world today. However, the way that I thought about it was completely backwards. I was hoping for a way to eliminate the autoimmune diseases that have been created as a result of the level of cleanliness that the US society has achieved. However, there was an important point I was missing. No matter how hard humans try and eliminate death, there will always be a trade off. Death is inviatble, and embracing it is the only thing we can do as a society. On the one hand, we have deaths due to a dirty environment. On the other, we have the side effects of having an environemtn so clean that our immune systems become weaker the less and less they are exposed to foreign pathogens. Nonetheless, we have a tradeoff that society is forced to accept. Yes humans are being killed and attacked by autoimmune diseases, but the situation was far worse 50 years ago. Death rates have decreases significantly, and our society is seeing an increase in the average lifespan. There is a fundamental trade off that is forced upon as, as we transition into our new era as a cleaner race that is more aware of its surroundings.

I had hypothesized that the issue in autoimmune hemolytic anemia dealt with complement, but now I strongly believe it is the SIAE gene cluster. Sialic acid is a sugar that is found on the surface of many cells in our body. Sialic acid is a very important sugar found on the surface of most of our cells that allows our body to distinguish it as foreign to our immune system. If not recognized, the body can engage the immune system to launch an attack against this foreign invader. The SIAE gene is in charge of making the enzyme that produces sialic acid[6]. Thus, loss in the function of this gene leads to a malfunction in the enzyme,

which leads to a lack of sialic acid on the surface of cells throughout the body[6]. This can be detrimental to the health of any human being, because it can cause the body to lose sight of what is and what is not the body itself. One of the more prominent cells in our body, are red blood cells. Because red blood cells are found everywhere, they are also prone to being attacked by the body. This is what I believe is going on in the body.

Our immune system is like a castle. It serves to protect us, and to both keep foreigners out and keep our current population in check. In this scenario, B-cells would be the watchmen that recognize threats to the castle, and then call upon reinforcement; antibodies. Once the B-cells alert the antibodies, then the attack against the invaders begins. The attack on the invader can be range from being led by macrophages, monocytes, inflammation, and many other immune responses. Taking this comparison a step further, sialic acid would be a name tag that all of the townspeople within the castle wear to be deemed part of the castle. The one who makes these name tags is the Sialic Acid Acetylesterase. Nonetheless, my hypothesis thus considers a case where these name tags are not created, and all of the townspeople (red blood cells) are not being considered towns people and are being destroyed.
The results definitely brought me down a path that I did not expect, but I am filled with hope that one day humans will conquer autoimmune diseases.

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